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CARCINOGENIC AND ANTICARCINOGENIC SUBSTANCES AND NUCLEIC ACID CONSTITUENTS: THEIR INTERACTION MECHANISM BY RAMAN, IR, AND NMR SPECTROSCOPY

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The molecular mechanism of interaction between carcinogenic and anticarcinogenic substances with nucleic acids constituents is still to be defined. We have observed by vibrational (IR and Raman) and NMR spectroscopies that the interaction between dimethylsulfate with adenosine produces a change of tautomerism on the base with formation of a reactive tautomeric species, 1-methyladenosine (1MeAdo) (1).

In the literature the interaction of the anticarcinogenic substances is studied, at molecular level, only with the main tautomeric form of the bases; changes of the tautomerism of bases, following the reaction with carcinogens, are not considered. Recently, we have prepared the adduct between 1MeAdo (which is the main product of the reaction between adenosine and dimethylsulphate) (1) and anticarcinogen cis$Pt(NH_3)_2Cl_2$ (cis-Pt). The formation of different species is proved by the vibrational and NMR data (2).

In this work we discuss the spectroscopic behaviour of 1MeAdo - a highly reactive nucleoside system (1) - in aqueous and buffered physiological pH solutions, i.e. in the environments where the reaction with anticarcinogen compounds takes place.

1MeAdo was prepared according to the literature method (3). Raman spectra of this compound were recorded with a JASCO R-500 spectrometer equipped with a data processing unit, using 4880 cm^{-1} exciting radiation from an Ar^+ laser. The ^{13}C NMR spectra were run with a Bruker CXP-300 spectrometer working at 75.458 MHz. The buffer used was a 0,4 M aqueous solution of NH_4HCO_3.

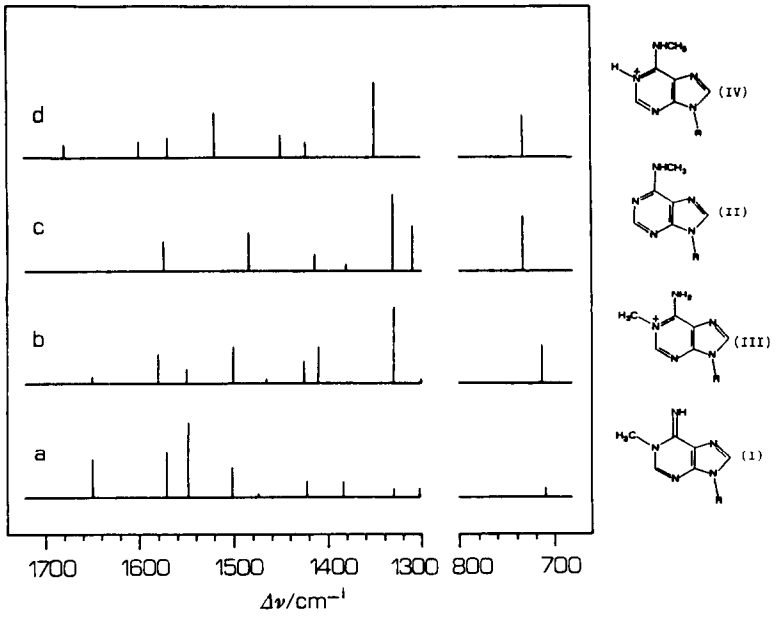

Fig.1 Raman spectra of:
a) 1MeAdo in DMSO (I);
b) 1MeAdoH$^+$ in DMSO (III);
c) N6MeAdo in D_2O (II);
d) N6MeAdoH$^+$ in D_2O (IV).

1MeAdo in aqueous solution shows, at the beginning, the 1650 ($\nu_{C6=NH}$), 1558, 1426, 720 (715 in D_2O) cm^{-1} bands typical of the iminic form (I) (fig.1a). After several days 1MeAdo shows a rather different spectrum in which the above mentioned bands disappear and those at 1576, 1489, 1342, 1315 and 742 (739 in D_2O) cm^{-1} characteristic of the rearranged form (II)(fig.1c), appear.

At intermediate times, there is an equilibrium between the two forms. The trend of the bands at 720 cm^{-1} (715 in D_2O) (for the iminic form) and at 742 (739 in D_2O) (for the rearranged form), can be used to evaluate the equilibrium. Initially, in aqueous buffered solution, the Raman spectrum of 1MeAdo shows both the bands typical of the iminic form (I) at 1650, 1558, 1426, 720 cm^{-1} and those of the aminic protonated form (III)(fig.1b) at 1573, 1415 and 1337 cm^{-1}; the 742 cm^{-1} band of the rearranged aminic form does not appear. Successively, 1MeAdo in buffered aqueous solution tends towards the rearranged aminic form II and the spectrum is coincident with that of the aqueous solution after a complete transformation. The bands at 1680, 1600, 1520, 1450 and 1355 cm^{-1} typical of the protonated rearranged form (IV) (fig.1d) are absent in the spectrum.

The ^{13}C NMR study (Tab.I) confirms the results derived from vibrational data and, moreover, gives a quantitative evaluation, as shown in the table, of the different forms at equilibrium by the integration of the ribose carbon resonances.

Table I. ^{13}C shifts[a,b] of iminic 1MeAdo (I) and its aminic N6methyl rearranged form (II) (D_2O and buffered physiological solutions) and % of I and II as a function of the time

solvent	compound	C_2	C_4	C_5	C_6	C_8	$-CH_3$
D_2O	I	149.8	142.5	122.9	155.9	139.4	36.6
"	II	153.1	147.8	119.9	155.6	140.5	28.2
buffer	I	149.4	145.4	121.5	153.8	142.0	37.6
"	II	153.4	148.2	119.2	155.7	140.5	28.4
	time	0'	20h	48h	96h	170h	
D_2O	I	100%	55%	30%	15%	0%	
"	II	0%	45%	70%	85%	100%	
	time	0'	5h	24h	260h		
buffer	I	100%	80%	40%	0%		
"	II	0%	20%	60%	100%		

Table II. ^{13}C shifts[a,b] of 1MeAdo in acidic (A), buffered physiological (B) and alkaline (C) solutions

compound	C_2	C_4	C_5	C_6	C_8	$-CH_3$
A	148.6	147.4	119.2	151.9	144.1	38.5
B	149.4	145.4	121.5	153.8	142.0	37.6
C	149.8	142.6	122.9	155.9	139.6	36.6

a) in ppm with respect to TMS as internal reference; b) the chemical shifts of the ribose residue are in agreement with the literature (4).

For aqueous (D_2O) as well as for buffered solutions the equilibrium goes from the iminic form (I) to the rearranged aminic one (II) and this last form is the only one observed after several days. Moreover, in aqueous buffered solution, the chemical shifts of the initial products do not correspond exactly to those of the iminic form of D_2O solution. These values are the average of the chemical shifts of 1MeAdo in acidic and basic environments (Tab.II). This fact confirms the Raman measurements and shows that initially, 1MeAdo is present in physiological pH buffered solution in a rapid equilibrium of its iminic form (I) and its aminic protonated form (III).

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